Identification of Transcriptional Regulator Effectors in *Burkholderia xenovorans LB400*

Tinh T. Nguyen[1], Ricardo Martí-Arbona[1], and Richard S. Hall[1], Tuhin Maity[1], John Dunbar[1], Clifford J. Unkefer[1] and Pat J. Unkefer[1]

Short Abstract — **The identification of transcription factor effectors for unknown regulators is directed by a combination of technologically advanced methods including bioinformatics and FAC-MS. Protein sequence alignments placed the TF Bxe_B2842 as a member of the MarR family of regulators, which is known to bind aromatic compounds containing phenolic rings. Screening of several aromatic compounds containing the aforementioned functional groups identified 2-aminophenol, an intermediate in the degradation of naphthalene and anthracene, as the tightest binder to Bxe_B2842. The gene context of the TF Bxe_B0878 suggests that it may participate in the regulation of genes in the aromatic amino acid biosynthesis, proline/arginine metabolism and/or lysine biosynthesis.**

***Keywords* — Transcription factor, effector, *Burkholderia xenovorans LB400*, FAC-MS, Bxe_B2842, Bxe_B0878**

I. BACKGROUND

Transcription factors (TFs) are primary control of protein production in every organism through gene expression. Phenotype and gene control occurs as a response to stimuli, which emerge from the binding of transcription factor effectors (TFEs), which cause the TRs to bind to a gene's promoter to either facilitate or inhibit transcription [1]. The most common TFs types are identified by their DNA-binding motif. Finding TFEs has proved to be a formidable task; several techniques have been applied to attempt their accurate identification, but without a significant break-through. Taking advantage of the success of affinity chromatography with MS detection (FAC-MS) [2] in drug discovery and the sensitivity of mass spectrometry have allowed us to develop a parallel approach to TFEs discovery, ligand ranking and dissociation studies.

II. METHODS

In our FAC-MS approach, TFs are immobilized on an affinity chromatography columns and a mixture of metabolites and possible ligands is continuously infused through the column and into an electrospray mass spectrometer. TFs are expressed with GST tag for immobilization onto a GSTrap column. A Thermo Exactive mass spectrometer is used to monitor the elution of the compounds from the FAC column in both positive and negative ion mode. The high sensitivity of the Exactive Orbitrap mass analyzer provides a ppm mass accuracy, facilitating the assignment of molecular formulas and furthermore, the identification of the potential TFEs.

III. RESULTS & CONCLUSION

The transcription factor Bxe_B2842 was screened with available compounds containing the phenolic functional group, and 2-aminophenol was found to be the tightest binder. This compound is an metabolite in the naphthalene and anthracene degradation pathways. Screening of Bxe_B0878 against compounds in the aromatic amino acid biosynthesis and proline metabolism indicated that proline and its derivatives are possible effector candidates. The predicted effectors 1-pyrroline-5- carboxylic acid, 1-pyrroline-3-hydroxy-5-carboxylic acid, 2,3-dihydrodipicolinic acid and 2,3,4,5-tetrahydrodipicolinic acid are not commercially available and are being synthesized in the lab.

In conclusion, FAC-MS has found to be a useful technique for high throughput screening of metabolites for the identification of TFEs. Because the binding of a metabolite from a pathway is frequently the stimulus and controls the TF, the identification of TFEs yields important insights about the metabolic pathways being regulated.

Acknowledgements: This work was funded by Los Alamos National Lab LDRD Program.

[1]Bioscience Division, Group B-8, LANL, PO Box 1663. E-mail: nguyen_t@lanl.gov, rm-a@lanl.gov, rshall@lanl.gov, tuhin@lanl.gov, dunbar@lanl.gov, cju@lanl.gov, punkefer@lanl.gov.

REFERENCES

[1] Changeux, J-P, Edelstein, S. J. (2005) Allosteric Mechanisms of Signal Transduction. *Science* **308**: 1414-1428.

[2] Slon-Usakievicz, J. J., Ng, W., Dai, J-R., Pasternak, A., Redden, P. R. (2005) Frontal affinity chromatography with MS detection (FAC-MS) in Drug Discovery. *Drug Discovery Today* **10**: 409-416.